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ACYL MIGRATION IN RIBONUCLEOSIDE DERIVATIVES

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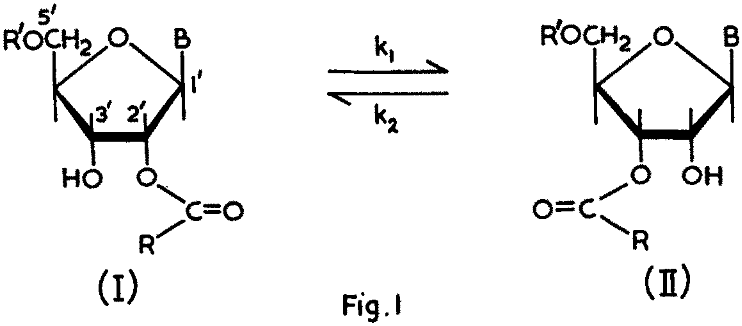
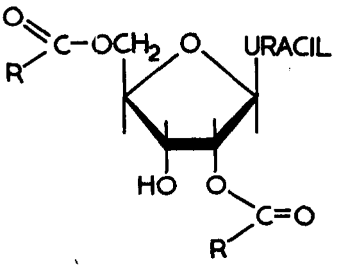


Fig.1

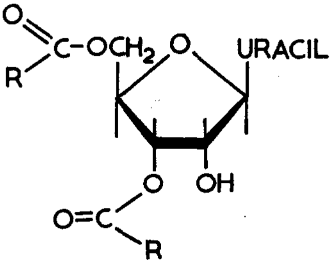
It is well known that monoacyl derivatives of certain 1,2-diol systems can isomerize by acyl migration (1 - 3). The earlier literature (4, 5) in the ribonucleoside field suggested that 2'-O-acyl derivatives (I) were unstable with respect to their 3'-isomers (II), but gave little indication that the reverse might be true. It was thus implied that the equilibrium constant, k_1/k_2 (see Fig. 1) was appreciably greater than unity. Recent work concerning the position of attachment of the aminoacyl group to the terminal adenosine residue in aminoacyl-s-RNA has involved orientation studies on mixtures of 2'- and 3'-aminoacyladenosines (6 - 9), obtained both by synthesis (6 - 10) and from biological sources. This work has led, in most instances[†], to an estimated value of ca. 2 for the equilibrium constant.

[†] Zachau and co-workers (6, 7) claimed that aminoacyladenosine, obtained by pancreatic ribonuclease digest of aminoacyl-s-RNA, was over 90% 3'-isomer.

Most of the previous work in this field has lacked clarity in two main respects. First, pure isomers of definite orientation were not used in the migration studies and secondly, no procedure had been developed for assaying mixtures of 2′- and 3′-isomers as stable derivatives, prepared under non-isomerizing conditions. We have developed (11) a general synthesis of 2′, 5′-di-O-acyluridines (III), and also a method of estimating mixtures of isomers (III and IV) with reasonable accuracy and under conditions where the rate of acyl migration is negligible. We now wish to report on the application of this work to the study of acyl migration.



(III)



(IV)

Before considering quantitative data relating to equilibrium and rate constants, it is relevant to discuss the partial acetylation of 5′-O-acetyluridine (4). When the latter compound was treated with a slight excess of acetic anhydride in pyridine solution, the products worked up and then chromatographed on silicic acid, a crude diacetate fraction was obtained in ca. 35% yield. A solution of this material in absolute ethanol slowly deposited pure crystalline 3′, 5′-di-O-acetyluridine (IV; R=Me) in nearly quantitative yield (12). However, when the crude diacetate fraction was examined by the mesylation procedure (11), it was found to contain the 3′, 5′- and the 2′, 5′-isomers in the respective proportions[‡] of 1.57:1. These observations

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[‡] $4\mathrm{x}10^{-5}$ mole of diacyl nucleoside was used per analysis. Electrophoretograms were run in duplicate and the results averaged. When the proportion of 3′, 5′- to 2′, 5′-isomer was in the region 0.5-2.0, the estimate was accurate within ±5%.

could be rationalized if it were assumed that as the 3′, 5′-diacetate crystallized, its concentration in solution was maintained by isomerization of the 2′, 5′-diacetate. This assumption was supported by the discovery that 2′, 5′-di-O-acetyluridine (III; R=Me) had been 53% isomerized to (IV; R=Me) after it had been heated in absolute ethanol solution, under reflux, for 4½ hr. Isomerization of the 2′, 5′-diacetate occurred slowly in ethanol solution at room temperature.

Anhydrous pyridine was found to be a particularly suitable solvent for acyl migration studies. As mesylation was conducted in pyridine solution, its use simplified the analytical procedure and, more importantly, made de-acylation of even the most labile esters unlikely. After a solution of 2′, 5′-di-O-acetyluridine (III; R=Me) in anhydrous pyridine had been allowed to stand at 20° for 24 days, analysis of the products showed that the proportion of 2′, 5′- to 3′, 5′-diacetate was 1:1.63. When 3′, 5′-di-O-acetyluridine (IV; R=Me) was treated in the same way, the corresponding ratio was found to be 1:1.79. It seemed that, within the limits of experimental error, these conditions were very nearly equilibrating and that the equilibrium constant, k_1/k_2 (see Fig. 1) at 20° was 1.7. After pyridine solutions of the same 2′, 5′- and 3′, 5′-diacetates had been heated under reflux for 2½ hr., the corresponding ratios were found to be 1:1.53 and 1:1.66 respectively. This led to an estimate of 1.6 for the equilibrium constant at 115°.

It was found that acyl migration proceeded at a conveniently measurable rate at 60° in anhydrous pyridine solution. Although consistent values could be obtained for equilibrium constants, it was not possible to obtain reproducible rate measurements. This was not surprising as the rate of equilibration of 3′, 5′-di-O-acetyluridine was doubled by the addition of as little as 0.1% water to pyridine. If it was assumed that acyl migration in anhydrous pyridine solution was kinetically a first order reversible reaction (13), then the rate of equilibration (k_1+k_2) of 2′, 5′-di-O-acetyluridine was found to be 12×10^{-5} $\sec^{-1}$ (see Table 1). Although this rate constant may only be accurate within a factor of two, it is reliable enough for comparative purposes.

TABLE 1

Equilibrium and Rate Constants in Anhydrous Pyridine Solution at 60°

Substrate	k_1/k_2	$10^5(k_1+k_2)$ $\sec^{-1}$
2',5'-di-O-benzoyluridine (III; R=Ph)	1.42	0.67
2',5'-di-O-acetyluridine (III; R=Me)	1.61	12
3',5'-di-O-formyluridine (IV; R=H)	2.00	450

It can be seen from Table 1 that the equilibrium constants for the dibenzoates, diacetates and diformates are all greater than one, but that the 3',5'-esters are only marginally more stable than their 2',5'-isomers. However, the rates of equilibration are strikingly different and this difference is well outside the limits of experimental error. This information is of considerable practical importance in oligoribonucleotide synthesis[†] and is also relevant to the problem of the orientation of aminoacyl-s-RNA.

The rate of migration of the formyl group in 3',5'-di-O-formyluridine (IV; R=H) is of special interest in the latter connection. The rate of hydrolysis of a mixture of 2'- and 3'-O-formyladenosines (14) in pH 7 buffer solution corresponded (15) to that of glycyl-s-RNA and was considerably greater than that of valyl-s-RNA. From recent work (8, 9) it appears

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[†] For example, 2',5'-di-O-p-anisoyluridine (III; R=p-MeO-C_6H_4-) was only 5% isomerized after 180 hr. in anhydrous pyridine solution at 20°. As phosphorylation can be effected in ca. 1hr. when mesitylenesulphonyl chloride is used as condensing agent (16), p-anisoyl would probably be a suitable 2'-protecting group. However, if required, an even less mobile acyl group could be found.

that, although acyl migration is much faster than solvolysis[‡], the ratio of the rate constants remains the same at different pH's. This being so, 2′(3′)-O-formyl nucleosides should be good model compounds for acyl migration studies involving the most sensitive aminoacyl-s-RNA systems.

The mesylation analysis procedure (11) can only be used to estimate the composition of mixtures of isomeric 2′- and 3′-derivatives of uridine[*]. Although for a given reaction medium, the rate of acyl migration probably depends largely on the nature of the acyl group, it would be desirable to have an orientation and assay procedure which was applicable to all 2′- and 3′-acyl ribonucleosides. We hope soon to report on such a general method.

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[‡] As has been noted elsewhere (17), when 3′,5′-di-O-acetyluridine (IV; R=Me) was heated in a 0.03 M solution of morpholine in ethanol at 50°, it was solvolyzed to give 5′-O-acetyluridine. The half-life of the reaction was *ca.* 4 hr. We found that a negligible amount of solvolysis had occurred after 5 min at 40°, but 36% of the starting material had been converted to the 2′,5′-diacetate (III; R=Me). Morpholine (and also pyridine in ethanol solution) exerts a marked catalytic effect on acyl migration.

[*] It may be possible to develop a similar method for cytidine derivatives (18).

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